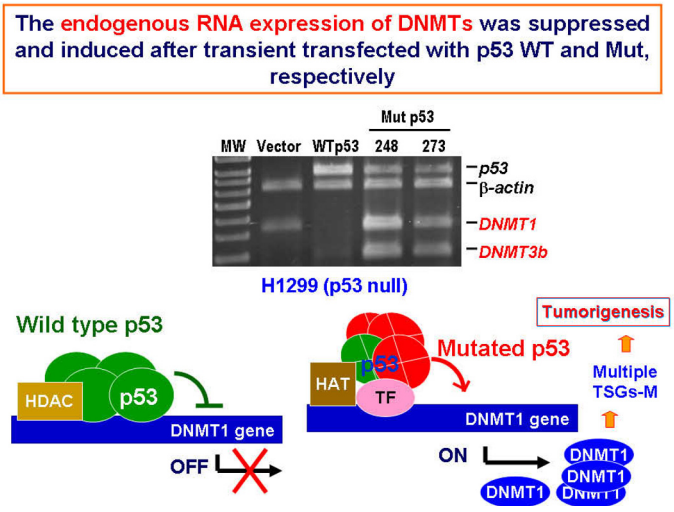
remains unclear. We performed DNMT1 promoter luciferase assay and found that wild type p53 could negatively regulate the DNMT1 promoter, whereas mutant p53 could activate DNMT1 promoter activity. The physical binding of wild type p53 and mutant p53 to DNMT1 promoters was identified by chromatin immunoprecipitation. These data suggest that deregulation of DNMTs is associated with the loss of transcriptional repression of p53. The p53 mutation results in overexpression of DNMT1 and hypermethyaltion of target tumor suppressor genes and ultimately leads to tumorigenesis.



C6-06 Cancer Genetics, Wed, 10:30 - 12:15

Gene expression signatures associated with lung adenocarcinomas in female non-smoker Chinese patients

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Female-non-smokers with lung adenocarcinomas is becoming prominent among Asian patients with lung cancer. The clinical parameters of gender, smoking habits, tumor histology, ethnic origin and EGFR mutation status were identified as predictors of clinical response to targeted therapy. The aim of this study was to identify the gene expression profiles of lung adenocarcinomas compared to normal lungs, with respect to gender difference, smoking habits and tumor EGFR mutation status among Hong Kong Chinese patients with lung adenocarcinomas. Total RNA extracted from 49 lung adenocarcinomas and 9 normal lung tissues and hybridized onto Affymetrix GeneChip HG-U133 Sets. Data were normalized and subjected to repeated iterations of unsupervised hierarchical clustering, with independent validation microarray data sets, for identification of stable sample clustering, followed by supervised analysis with Significance Analysis of Microarray (SAM) and Support Vector Machine (SVM) for identification of molecular classifiers with respect to sample clustering results. Unsupervised hierarchical clustering revealed stable clustering of adenocarcinomas vs normal lungs, female-non-smokers vs other tumors, and tumors bearing EGFR mutation at exons 18 - 21 vs wildtype tumors. Supervised analysis identified discriminatory gene classifiers that predict lung adenocarcinomas (gene classifiers = 27, prediction sensitivity and specificity of 100%), female non-smokers (gene classifiers = 22, prediction sensitivity of 65 - 85% and specificity of 84 - 94% with different independent validation sets) and EGFR mutation status (gene classifiers = 26, prediction sensitivity and specificity of 100%). Our data suggested that there is a group of Chinese female non-smokers with lung adenocarcinomas which displays gene expression profiles distinct from pulmonary adenocarcinomas from patients with other clinical characteristics. The identification of gene classifiers could provide information for further study on lung adenocarcinomas, female non-smokers with lung cancer and lung tumors bearing EGFR mutations.

C6-07 Cancer Genetics, Wed, 10:30 - 12:15

Mutations in the LKB1 tumor suppressor are frequently found in tumors from Caucasian NSCLC patients

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Background: Peutz-Jeghers syndrome has been associated with mutations in the LKB1 tumor suppressor gene. Somatic mutations of LKB1 gene have been found in human cancers including non-small cell lung cancer (NSCLC) with adenocarcinoma histology. Reports with small number of cell lines and tumors have suggested the prevalence of LKB1 mutations in NSCLC to be ~30%. Relation of LKB1 mutations to the patient gender, age, smoking history, tumor stage and outcome, and their relationship to other commonly mutated genes in NSCLC is unknown.

Methods: NSCLC tumor specimens (n = 167) with adenocarcinoma (n = 161) and adenosquamous carcinoma histologies (n = 6) were collected from surgical resections and RNA was extracted using routine methods. Some of the specimens (n = 63) were collected in Korea, while rest of the specimens (n = 104) came from Caucasian patients from the US. Tumors were screened for LKB1 mutations using the SURVEYOR-WAVE method. In SURVEYOR-WAVE method, the whole coding region of LKB1 gene was PCR amplified in two overlapping amplicons, PCR products were digested with SURVEYOR-enzyme which cleaves mismatches, and the specimens were analyzed with sensitive HPLC method for shorter digestion products. If digestion products were present, the specimen was sequenced using Sanger method to verify the mutation. Mutational analysis K-Ras, B-Raf, and EGFR hotpots were done either by SURVEYOR-WAVE method analogously to LKB1 gene analysis or by direct sequencing with Sanger method. Statistical comparisons were done using Fisher's exact test, p values < 0.05 were considered significant

Results: Mutations of the LKB1 gene were detected in twenty-two tumors (13%). Interestingly, twenty of the LKB1 mutations were detected in specimens collected in U.S. (prevalence 19%) while only two mutations (prevalence 3%) were detected in specimens from Asian origin (p = 0.004). The mutations were mainly deletions and insertions (82%) but some missense (9%), and nonsense (9%) mutations were also found. No correlation of LKB1 mutations to gender, age, or stage

was detected but there was tendency for LKB1 mutations occuring in smokers vs. non-smokers ($p = 0.29$). Furthermore, the outcome of stage I-II NSCLC patients treated with surgery alone did not differ based on LKB1 mutation status. K-Ras mutations were detected in thirty-two (19%) of tumor specimens with seven (21% of the K-Ras mutants) of these occurring concurrently with LKB1 mutations which, however, was statistically insignificant ($p = 0.14$). Three B-Raf mutations were detected but none of these occurred concurrently with LKB1 mutations. Twenty-eight EGFR mutants (17%) were found with only one of them occurring concurrently with LKB1 mutation, which was missense outside the kinase domain of LKB1 ($p = 0.13$).

Conclusions: Mutations of LKB1 gene occur frequently in NSCLCs from Caucasian origin. There is tendency of coexistence in LKB1 and K-Ras mutations and exclusivity in LKB1 and EGFR mutations but these were statistically insignificant with our limited tumor material. Further studies are validated to clarify the outcome of LKB1 and K-Ras double mutation NSCLC patients and effect of LKB1 mutations to therapeutic responses in NSCLC.

Session C7: Tumor & Cell Biology

Wednesday, September 5

C7-02 **Tumor & Cell Biology, Wed, 10:30 - 12:15**

Bronchial preneoplasia: MDM2 overexpression occurs before p14ARF and NPM aberrant expressions

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Background: p53 intracellular poll is regulated by both MDM2 induced degradation and p14ARF inhibition of MDM2-p53 complex formation. It was commonly accepted that p14ARF inhibits MDM2-p53 complex by sequestering MDM2 in nucleoli. However in recent models, nucleophosmin (NMP) might sequester p14ARF in nucleoli preventing its inhibition of nucleoplasmic MDM2.

We studied whether MDM2, p14ARF and NPM expressions are modified during early squamous carcinogenesis, the time course of appearance of these abnormalities and relative intracellular localizations of these proteins.

Methods: 179 biopsies, including normal tissue, all type of bronchial preneoplastic lesions and early lung cancer were assessed for MDM2, p14ARF and NPM expressions using immunohistochemistry. 132 biopsies were evaluable for all three markers.

Abnormal expression was defined as follows: for MDM2, increased expression based on a score including intensity and number of positive cells (Eymin, 2002), for p14ARF, expression <10% or relocalization from the nucleoplasm to nucleoli in ≥10% of cells, for NMP, disappearance from the nucleoplasm and concentration in nucleoli in ≥10% of cells.

Triple immunofluorescent staining with antibodies directed against p14ARF, MDM2 and p53 (13 biopsies) and p14ARF, MDM2 and NPM (11) were performed, followed by laser scanning confocal microscopy.

Results:

1) Expression

Aberrant expression was observed for all three proteins in lung preneoplastic lesions.

Protein	Type of abnormality	Histo-pathological cut-off	Number of biopsies with aberrant expression	Number of biopsies with aberrant expression	p
			< cut-off	= or > cut-off	
MDM2	overexpression	mild dysplasia	22/94 (23%)	67/85 (79%)	< 0.001
NPM	relocalization to nucleoli	moderate dysplasia	4/75 (5%)	16/61(26%)	0.034
p14ARF	loss of expression (1)	severe dysplasia	19/100 (19%)	23/44 (52%)	0.013
p14ARF	relocalization to nucleoli (2)	severe dysplasia	5/81 (6%)	8/21 (38%)	< 0.001
p14ARF	aberrant expression (1) or (2)	severe dysplasia	24/100 (24%)	31/44 (70%)	< 0.001

Aberrant p14ARF expression was statistically associated with MDM2 overexpression: 75% and 85% of biopsies showing p14ARF loss or relocalization overexpressed MDM2.

2) Localization

Immunohistochemistry indicated that relocalizations of NPM and p14ARF to nucleoli were associated ($p<0.001$): 77% of biopsies expressing relocalized p14ARF showed relocalized NPM.

Immunofluorescent staining indicated that MDM2 and p53 were localized in the nucleoplasm of bronchial epithelial cells, excluding nucleoli.

Three patterns were observed for p14ARF: 1) not detected, 2) expressed either in nucleoli, 3) either in the nucleoplasm alone.

Two patterns were observed for NPM: 1) present in nucleoli only, in which NPM colocalized with p14ARF but not with MDM2, 2) present both in nucleoli and in the nucleoplasm, in which NPM colocalized with nucleoplasmic p14ARF.

Conclusions: Our data suggest that MDM2, NPM and p14ARF could all play an active role early in lung squamous carcinogenesis: MDM2 is already aberrantly expressed at the grade of mild dysplasia. p14ARF loss of expression or its relocalization from the nucleoplasm to nucleoli was associated with increased MDM2 expression. Both NMP and p14ARF, but not MDM2, are relocalized to nucleoli, supporting the hypothesis that relocalization of p14ARF in nucleoli might impair p14ARF-MDM2 complex formation.